Original Article

Inhibition of the proliferation, invasion, migration, and epithelial-mesenchymal transition of prostate cancer cells through the action of *ATP1A2* on the TGF-β/Smad pathway

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Contributions: (I) Conception and design: B Zhang, A Ding; (II) Administrative support: A Ding; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: B Zhang, Z Zhu, X Zhang, F Li; (V) Data analysis and interpretation: B Zhang, Z Zhu, X Zhang, F Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: Prostate cancer (PC) is one of the major male malignancies worldwide. Because Na^+-K^+-ATPase is widely involved in various pathological processes, but the action of its α2 subtype (*ATP1A2*) in PC is unclear, we investigated the role of *ATP1A2* in the invasion and migration of PC cells.

Methods: We measured the expression levels of *ATP1A2* in human normal prostate epithelial cell line (RWPE-1) and PC cell lines (PC-3 and DU145) by quantitative real-time PCR (qRT-PCR) and western blot. Cell proliferation, apoptosis, migration, and invasion of PC-3 and DU145 cells were investigated through clone formation assay, EdU assay, flow cytometry and transwell assay, respectively. The effect of *ATP1A2* on a tumor-inhibitory pathway [transforming growth factor-β (TGF-β)/Smad] was assessed using western blot. In addition, tumor formation was detected using *in vivo* xenograft model in male BALB/c nude mice.

Results: The Cancer Genome Atlas (TCGA) analysis showed that *ATP1A2* expression was reduced in PC patients ($P<0.05$), and patients with low *ATP1A2* expression had a lower survival rate ($P<0.05$). *ATP1A2* levels were significantly reduced in PC-3 and DU145 cells, compared with RWPE-1 cells ($P<0.01$). We also demonstrated that overexpression of *ATP1A2* significantly inhibited the proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) of PC-3 and DU145 cells ($P<0.01$) and promoted apoptosis ($P<0.01$). However, silencing *ATP1A2* had the opposite effect ($P<0.01$). In addition, overexpression of *ATP1A2* significantly inhibited the TGF-β/Smad pathway ($P<0.01$), whereas silencing *ATP1A2* activated the TGF-β/Smad pathway ($P<0.01$). Meanwhile, the effect of *ATP1A2* silencing on the proliferation, apoptosis, migration and invasion was reversed by TGF-β/Smad pathway inhibitor (LY364947). Furthermore, *ATP1A2* inhibited tumor growth *in vivo*.

Conclusions: *ATP1A2* inhibited proliferation, apoptosis, migration, invasion, and EMT in PC by inhibiting the TGF-β/Smad pathway.

Keywords: *ATP1A2*; prostate cancer cell (PC cell); transforming growth factor-β/Smad (TGF-β/Smad); invasion; migration

Submitted Oct 31, 2021. Accepted for publication Jan 07, 2022.
doi: 10.21037/tau-21-1117
View this article at: https://dx.doi.org/10.21037/tau-21-1117

Introduction

Prostate cancer (PC) is a relatively common malignancy of the male genitourinary system, particularly affecting middle-aged and older men, and has a high incidence in Europe and the USA (1), with an increasing incidence in Asian countries in recent years (2). PC is highly aggressive and prone to metastasis (3), which is the cause of almost all deaths of PC patients and occurs mostly in the late stage of the disease, is usually resistant to various treatment methods and has a 5-year survival rate of >30% (4,5). Therefore, it is particularly urgent to understand the mechanism of the occurrence and development of PC and to find new and effective diagnostic techniques and treatments for PC.

The well-studied sodium pump (Na^+-K^+-ATPase) is widely involved in various pathological processes (6). It has different subtypes, including four different subtypes of the α subunit, which all have different functions. As an example, α1, or ATP1A1, is considered to play a tumor-promoting role in malignant gliomas, small cell lung cancer, and other tumors (7,8); α3 (*ATP1A3*) is considered to have the ability to promote metastasis of colon cancer (9); α4 (*ATP1A4*) exists only in testicular tissue (10); and repeated mutations in *ATP1A2* have been found to be involved in the biological processes of lung cancer cell metabolism, division, death, and immune regulation (11). Moreover, the data of The Cancer Genome Atlas (TCGA) have demonstrated that *ATP1A2* expression is markedly reduced in PC tissues, and low *ATP1A2* expression in patients is associated with a lower survival rate. However, there are few published studies of the relationship between α2 (*ATP1A2*) and PC. Therefore, the purpose of this study was to measure the expression of *ATP1A2* in PC and explore its effect on the invasion and migration of PC cells in two cell lines (PC-3 and DU-145), with the goal of expanding our knowledge of the role of Na^+-K+-ATPase in tumors and to provide new ideas for the treatment of PC in clinical practice.

The ligand, receptor, and intracellular signal transduction molecule Smad protein of the transforming growth factor-β (TGF-β) signal transduction pathway forms a tumor-inhibitory pathway (12,13). Abnormality of any element in the pathway can cause signal transduction disorders and lead to tumorigenesis. Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose their epithelial phenotype and acquire a stromal phenotype, which then transforms into stromal cells (14). As an important part of the deterioration and metastasis of PC, the activation of EMT is regulated by a series of gene expression changes. Previous studies have found that the TGF-β1/Smad pathway regulates the expression of Snail and Twist1 (15). An abnormally activated TGF-β1/Smad pathway mediates EMT by inducing Snail1 overexpression in most tumors (16). The binding of TGF-β1 to the TGF receptor on the cell surface leads to phosphorylation of TGF-β RII and further activates Smad3. P-Smad3 enters the nucleus to initiate the expression of Snail1 and Twist (17). Basic studies have found that the EMT of tumor cells can be reversed by inhibiting the TGF-β/Smad pathway.

In this study, we for the first time revealed the expression level and effect of *ATP1A2* in PC and unraveled that *ATP1A2* played significant roles in PC through the regulation of TGF-β/Smad pathway. We present the following article in accordance with the ARRIVE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-21-1117/rc).

Methods

Cell culture and treatment

Human PC cell lines (PC-3, VCaP, LNCaP and DU145) and human normal prostate epithelial cell line (RWPE-1) were acquired from the American Type Culture Collection (ATCC, USA). The cells were cultured in complete culture medium containing 10% fetal bovine serum (Dulbecco's Modified Eagle Medium) at a constant temperature of 37 ℃ in a 5% CO_2 incubator. Cells in the logarithmic growth phase and in good condition were selected for the experiment.

The constructs pcDNA-*ATP1A2* and si-*ATP1A2* and their negative controls were transiently transfected into PC-3 and DU145 cells using Lipofectamine® 3000 Reagent (Invitrogen, USA) according to the manufacturer's instructions. The transfected PC-3 and DU145 cells were divided into five groups: (I) pcDNA-NC group (treated with pcDNA-NC), (II) pcDNA-*ATP1A2* group (treated with pcDNA-*ATP1A2*), (III) si-NC group (treated with *ATP1A2* siRNA negative control), (IV) si-*ATP1A2* group (treated with *ATP1A2* siRNA) and (V) si-*ATP1A2* + LY364947 group (treated with *ATP1A2* siRNA and LY364947). LY364947, an inhibitor of the TGF-β/Smad pathway, was obtained from Abcam, UK. All cells were then cultured in a 37 ℃ incubator for 48 h. pcDNA-NC, pcDNA-*ATP1A2*, si-NC, and si-*ATP1A2* were supplied by GenePharma (Shanghai, China).

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed to analyze *ATP1A2* expression as previously described (18,19). Total RNA from cells was extracted using TRIzol reagent. After concentration and purity detection, cDNA was synthesized with FirstStrand cDNA Synthesis Supermix (Transgen Biotech, Beijing, China) according to the manufacturer's instructions. The expression of *ATP1A2* was determined through qRT-PCR using an ABI 7500 system (Applied Biosystems, USA), with Green qPCR Supermix (Transgen Biotech, Beijing, China). The gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. GAPDH was used as an internal reference. The primers were supplied by GenePharma as follows:

ATP1A2 (sense): 5'-GGGCACAGATATGGTCCCTG-3', (antisense): 5'-TTGTCCGTCTGGGAGTTTCG-3'; GAPDH (sense): 5'-TTAGGAAAGCCTGCCGGTGA-3', (antisense): 5'-GCCCAATACGACCAAATCAGAGAAT-3'.

Colony formation

Cells in the logarithmic growth phase were inoculated into 6-well plates according to appropriate cell density, and were evenly dispersed and cultured in a cell incubator for 2 weeks. When cell colonies were observable in the well plate with the naked eye, the cells were fixed with methanol, stained with crystal violet, and counted directly.

EdU detection

Cells were seeded in a 96-well plate at a density of 5×10^3 cells/well and 200 µL/well. After the cells fused, they were incubated with 50 mmol/L EdU solution (Beyotime, Nantong, China) for 2 h, fixed with 4% formaldehyde and treated with 0.5% Triton for 20 min to permeabilize at room temperature. Staining of the cell nuclei was performed using 4',6-diamidino-2-phenylindole (DAPI; Sigma, USA). The prepared samples were photographed under a fluorescence microscope.

Cell apoptosis

The apoptotic ability of PC-3 and DU145 cells was analyzed using the Annexin-V-FITC cell apoptosis assay kit (Beyotime) following the manufacturer's protocols. After being resuspended in the binding buffer, the transfected PC-3 and DU145 cells were stained with Annexin V-FITC and propidium iodide in the dark for 15 min. The cells were then analyzed with a flow cytometer (FACScan, BD Bioscience, USA).

Transwell assay

After cells were digested and centrifuged, 800 µL of culture medium containing 10% serum was added to the lower chamber of transwell chambers (Corning, Corning, NY, USA), 100 µL cell suspension was added to the upper chamber, and the mixtures were cultured for 24 h in an incubator at 37 ℃. The chamber was then removed to allow the fluid in the upper chamber to dry out, the cells was fixed with 4% paraformaldehyde at room temperature for 30 min, and finally stained with crystal violet at room temperature for 30 min. The chamber was then gently rinsed with clean water and photographed under a microscope.

Cell invasion assay was firstly carried out using a Corning Polycarbonate Membrane Insert transwell chamber (8-µm pore size, Corning, USA) coated with Matrigel (BD Bioscience). The subsequent steps were as described above.

Western blot

Western blot was performed as previously described (20,21). Cell proteins were extracted with RIPA lysis buffer (KeyGene Biotech, Nanjing, China), and the protein concentration was determined by BCA (KeyGene Biotech). After SDS-PAGE electrophoresis and membrane transfer, the membranes were blocked with 5% non-fat milk for 1 h and then incubated with primary antibody overnight at 4 ℃. After incubation with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h, the membranes were visualized using enhanced chemiluminescence reagents (Beyotime). The primary antibodies used in the study included Bax, caspase-3, Bcl-2, E-cadherin, matrix metalloproteinase (MMP)-2, MMP-9, and β-actin (purchased from ProteinTech, IL, USA), anti-Snail, anti-vimentin, anti-N-cadherin, anti-β-catenin, anti-TGF-β, α-SMA, anti-p-Smad2, anti-Smad2, anti-p-Smad3, anti-Smad3, and anti-cleaved caspase-3 were purchased from Cell Signaling Technology (MA, USA). Anti-*ATP1A2* was supplied by Abcam (Cambridge, UK).

In vivo xenograft model

Ten male BALB/c nude mice (4 weeks old), obtained from Beijing Vital River, were injected subcutaneously in their

right dorsal flanks with 1×10^7 DU145 cells transfected with either pcDNA-NC or pcDNA-*ATP1A2*. Animal experiments were performed under a project license (No. KYKT2018-043-A1) granted by the Animal Ethics Committee of Affiliated Dongguan People's Hospital, Southern Medical University, in compliance with Affiliated Dongguan People's Hospital, Southern Medical University guidelines for the care and use of animals. Subcutaneous tumor volumes were measured every 6 days and calculated as: volume = length × width^2 × 1/2. On the 30th day after injection, the mice were killed humanely and the subcutaneous tumors were removed and weighed.

Bioinformatic analysis

The differential expression of *ATP1A2* in PC and normal tissues, and the prognostic analysis of PC patients were performed by Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia2.cancer-pku.cn) based on TCGA (https://cancergenome.nih.gov/).

Statistical analysis

The data were analyzed using SPSS 21.0, and the measurement data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Student's *t*-test or one-way analysis of variance (ANOVA) test. The difference was considered statistically significant at $P<0.05$.

Results

ATP1A2 expression in PC tissues and cells

The results of GEPIA based on TCGA database revealed that *ATP1A2* expression is markedly reduced in PC tissues (*Figure 1A*), and low *ATP1A2* expression in patients is associated with a lower survival rate (*Figure 1B*). Moreover in our study, the mRNA and protein expressions of *ATP1A2* were significantly reduced in PC cells compared with normal human prostate epithelial cells (*Figure 1C,1D*). Compared with the other PC cells, PC-3 and DU145 cells showed the lowest *ATP1A2* expression, which is why we selected them for the subsequent experiments.

Effect of ATP1A2 on PC cell proliferation

We transfected PC-3 and DU145 cells with pcDNA-*ATP1A2* and si-*ATP1A2* to evaluate the role of *ATP1A2* in cell proliferation. The mRNA and protein expressions of *ATP1A2* increased after transfection with pcDNA-*ATP1A2*, but decreased after transfection with si-*ATP1A2* (*Figure 2A,2B*). As shown in *Figure 2C*, *ATP1A2* overexpression reduced the number of PC-3 and DU145 cell clones. In contrast, *ATP1A2* silencing increased the colony number (*Figure 2C*). EdU staining also showed that proliferation of PC-3 and DU145 cells was inhibited by *ATP1A2* overexpression and promoted by *ATP1A2* silencing (*Figure 2D*). These data suggested that *ATP1A2* markedly inhibited the proliferation of PC cells.

Effect of ATP1A2 on apoptosis of PC-3 and DU145 cells

Overexpression and silencing of *ATP1A2* promoted or inhibited apoptosis in PC-3 and DU145 cells, respectively (*Figure 3A*). Western blot results revealed that overexpression of *ATP1A2* elevated the expression of Bax and cleaved caspase-3, and reduced the expression of Bcl-2 in PC-3 and DU145 cells, whereas *ATP1A2* silencing had the opposite effect (*Figure 3B*). These data demonstrated that *ATP1A2* promoted apoptosis of PC cells.

Effect of ATP1A2 on the migration and invasion of PC cells

Inhibition of tumor cell migration and invasion can effectively inhibit tumor metastasis. Overexpression and silencing of *ATP1A2* inhibited or promoted the migration and invasion of PC cells, respectively (*Figure 4A,4B*). Overexpression of *ATP1A2* significantly inhibited the expression of MMP-2 and MMP-9, whereas silencing of *ATP1A2* significantly promoted their expression (*Figure 4C*).

Effect of ATP1A2 on EMT in PC cells

Subsequently, we detected the expression of EMT-related marker proteins. Overexpression of *ATP1A2* significantly inhibited Snail, α-SMA, vimentin, N-cadherin, and β-catenin expression and promoted E-cadherin expression, whereas silencing of *ATP1A2* had the opposite result (*Figure 5A,5B*). These data revealed that *ATP1A2* could significantly inhibit EMT in PC cells.

Effect of ATP1A2 on the TGF-β/Smad pathway in PC cells

The TGF-β-Smad signaling pathway plays a key role in cellular EMT, which can reduce the adhesion between

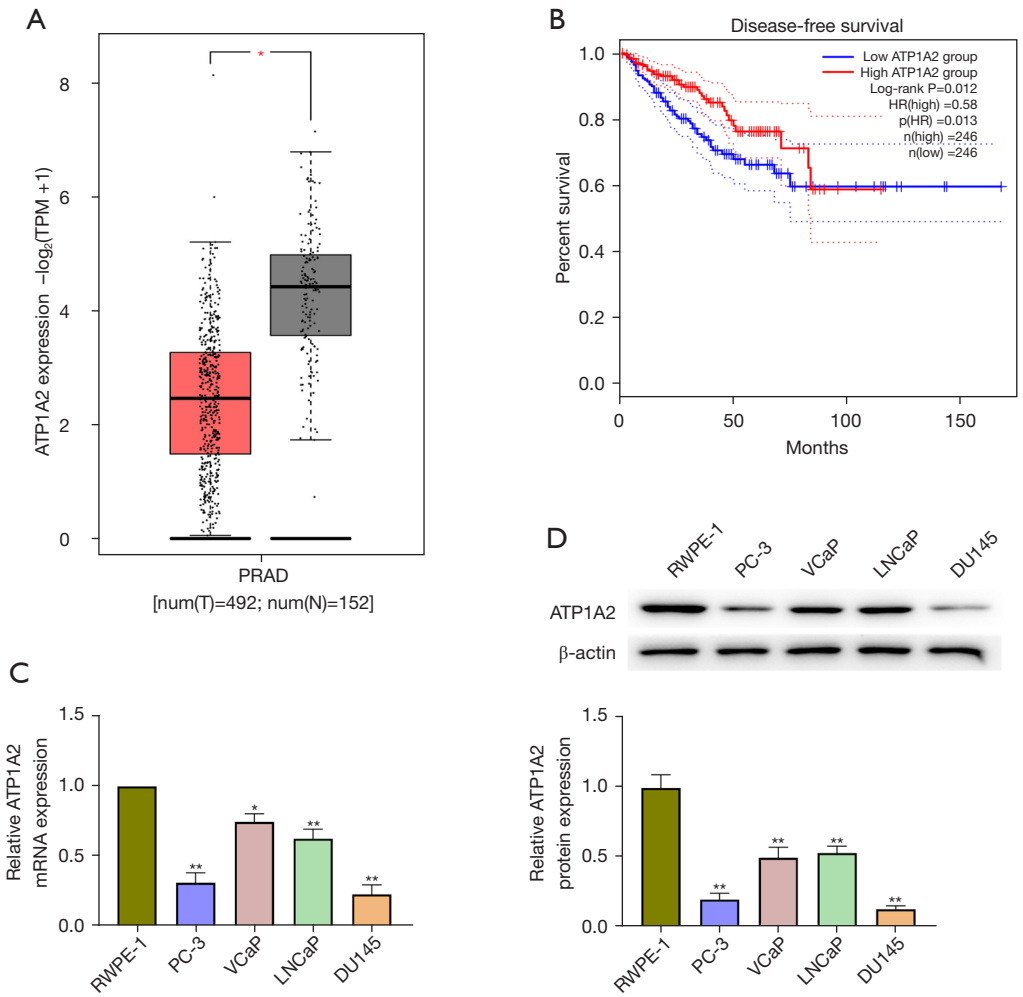

Figure 1 Downregulation of *ATP1A2* expression in PC tissues and cells. (A) *ATP1A2* expression analyzed by GEPIA based on TCGA database (n=152 for normal group, and n=492 for tumor group). (B) GEPIA based on TCGA database showing that patients with high *ATP1A2* expression had a higher survival rate (n=246 for *ATP1A2*-low group, and n=246 for *ATP1A2*-high group). (C) mRNA expression of *ATP1A2* in human normal prostate epithelial cells (RWPE-1) and PC cells (PC-3, VCaP, LNCaP and DU145) as detected by qRT-PCR. (D) Protein expression of *ATP1A2* in RWPE-1 and PC-3, VCaP, LNCaP and DU145 was detected by western blot. *, P<0.05; **, P<0.01. PC, prostate cancer; GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas; qRT-PCR, quantitative real-time PCR; PRAD, prostate adenocarcinoma.

cells, allowing tumor cells to penetrate the basement membrane, extensively invade adjacent tissues, and develop distant metastases. Therefore, we examined the effect of *ATP1A2* on the TGF-β/Smad pathway. As shown in *Figure 6A,6B*, overexpression of *ATP1A2* markedly inhibited the expression of TGF-β, p-Smad2, and p-Smad3 in PC-3 and DU145 cells, whereas *ATP1A2* silencing significantly promoted their expression. These data confirmed that *ATP1A2* inhibited the activation of the TGF-β/Smad pathway.

Effect of ATP1A2 inhibiting the TGF-β/Smad pathway

As seen in *Figure 7A*, the facilitating effect of *ATP1A2* silencing on the expression of TGF-β, p-Smad2, and p-Smad3 in PC-3 and DU145 cells was significantly weakened by LY364947 (an inhibitor of the TGF-β/Smad pathway). The effect of *ATP1A2* silencing on DU145 cell proliferation was reversed by LY364947 (*Figure 7B*), whereas the inhibitory effect of *ATP1A2* silencing on the apoptosis of DU145 cells was reversed by LY364947 (*Figure 7C*). Moreover,

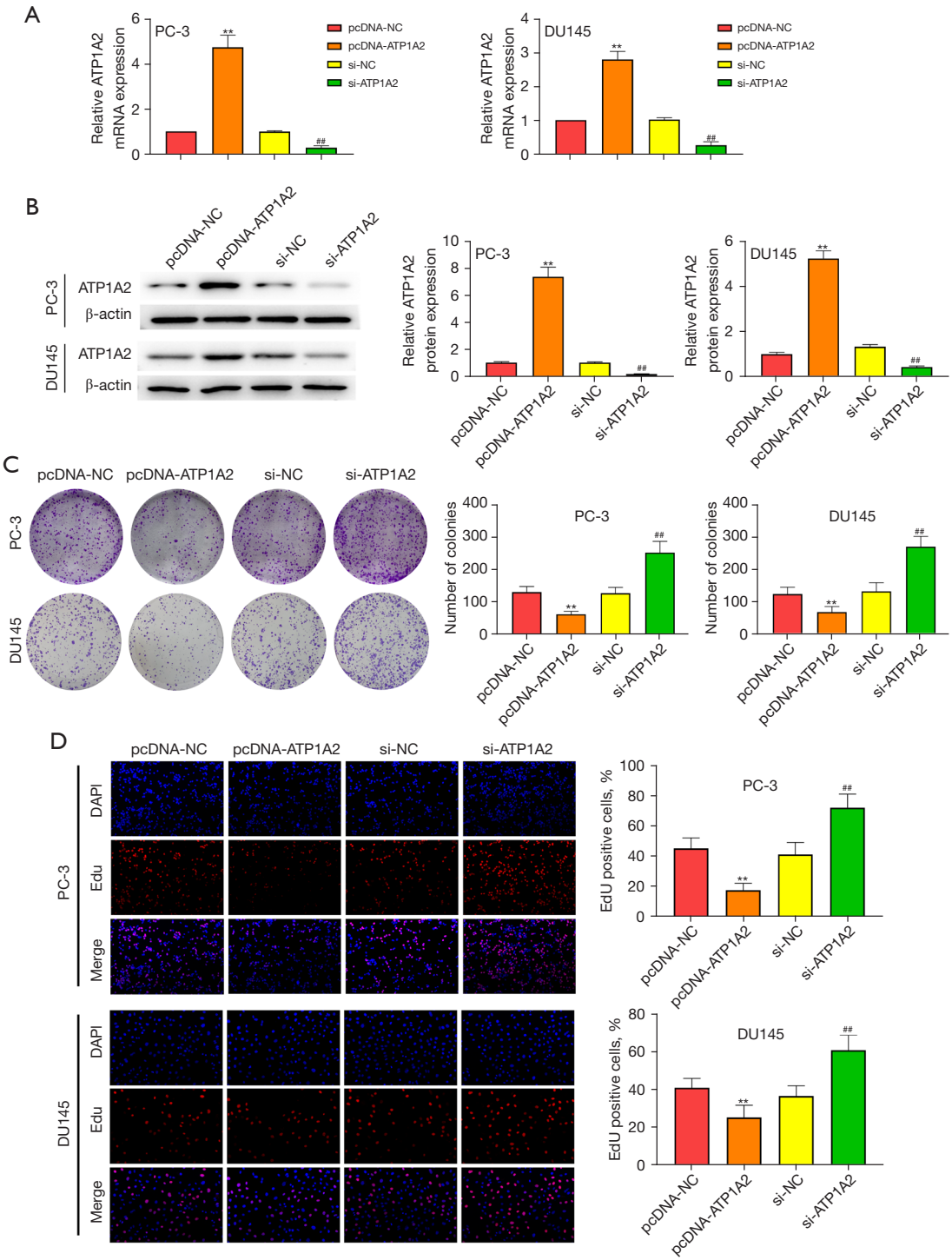

Figure 2 Effect of *ATP1A2* on proliferation of PC cells. (A) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the mRNA level of *ATP1A2* in PC-3 and DU145 cells was detected by qRT-PCR. (B) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the level of *ATP1A2* in PC-3 and DU145 cells was detected by western blot. (C) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the colony numbers of PC-3 and DU145 cells were determined by colony formation assay (stained with crystal violet; magnification 100×). (D) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the proliferation of PC-3 and DU145 cells was tested by EdU staining (magnification 200×). **, P<0.01; ##, P<0.01. PC, prostate cancer; qRT-PCR, quantitative real-time PCR.

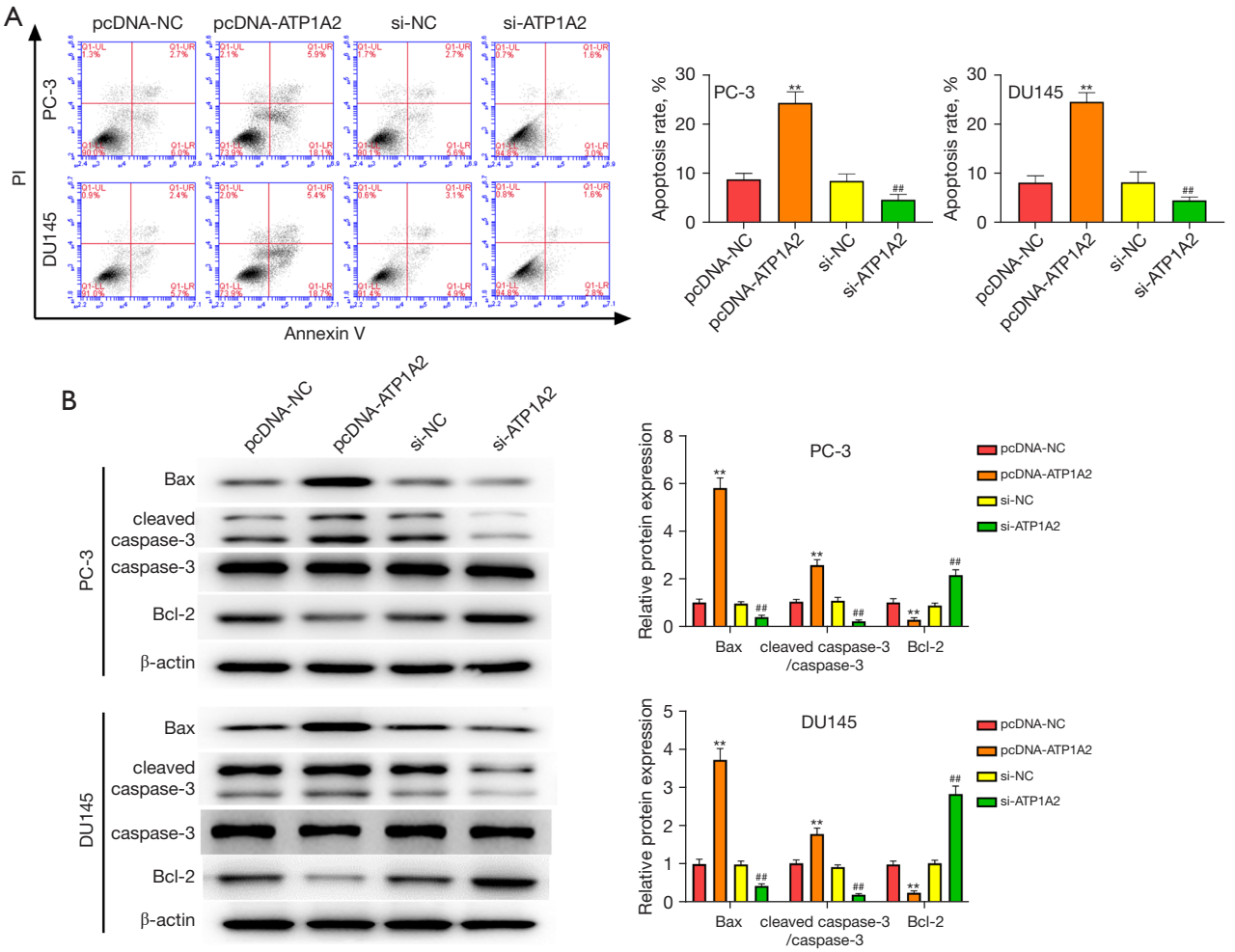

Figure 3 Effect of *ATP1A2* on apoptosis of PC cells. (A) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, apoptosis of PC-3 and DU145 cells was detected by flow cytometry (stained with Annexin V-FITC and propidium iodide). (B) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the expressions of Bax, cleaved caspase-3, caspase-3 and Bcl-2 in PC-3 and DU145 cells were measured using western blot. **, P<0.01; ##, P<0.01. PC, prostate cancer.

LY364947 reversed the effect of *ATP1A2* silencing on the migration and invasion of DU145 cells (*Figure 7D*). These results suggested that *ATP1A2* can suppress the proliferation, migration and invasion of PC cells, and promote their apoptosis by inhibiting the TGF-β/Smad pathway.

Effect of ATP1A2 on tumor growth in vivo

To explore the function of *ATP1A2* on tumor growth *in vivo*, the xenograft assay was performed (*Figure 8A*). Tumor volume and weight were significantly decreased in the pcDNA-*ATP1A2* group when compared with the pcDNA-NC group (*Figure 8B,8C*). These results revealed that *ATP1A2* overexpression can inhibit tumor growth *in vivo*.

Discussion

The symptoms of early PC are subtle, and the disease is difficult to diagnosis until it has progressed (22). Although some patients improve after treatment, many others progress to resistant PC, which leads to difficulties in treatment and poor prognosis; consequently the patient's quality of life is seriously affected (23,24). Therefore, finding more effective therapeutic targets is urgent.

As the target protein of cardiotonic glycosides, Na^+-

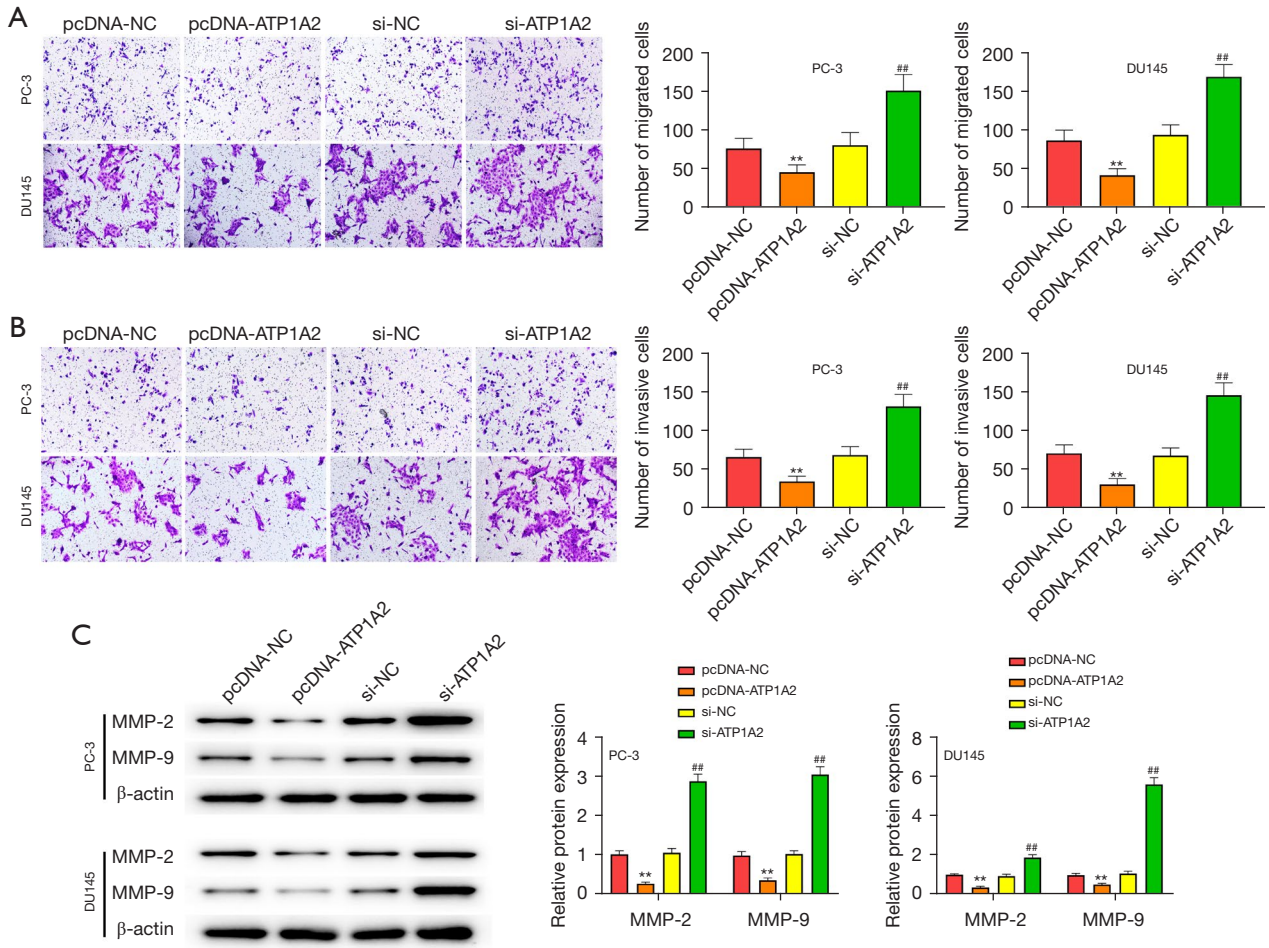

Figure 4 Effect of *ATP1A2* on the migration and invasion of PC cells. After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the migration (A) and invasion (B) of PC-3 and DU145 cells was detected by transwell assay (stained with crystal violet; magnification 200×). (C) After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the expressions of MMP-2 and MMP-9 in PC-3 and DU145 cells were determined using western blot. **, P<0.01; ##, P<0.01. PC, prostate cancer; MMP, matrix metalloproteinase.

K^+-ATPase has been widely studied in the context of heart failure, arrhythmia, and other diseases; its signal transduction function has been gradually discovered and recognized by researchers, and its role in tumorigenesis is now a major focus (25). *ATP1A2* is mainly expressed in the adult heart, vascular smooth muscle, skeletal muscle, brain, adipocytes, cartilage, and bone, but less attention has been given to *ATP1A2* in tumors because of its tissue specificity (26,27). In this study, we revealed that *ATP1A2* is expressed at low levels in PC tissues and cells, and that PC patients with low *ATP1A2* expression have a higher mortality rate. Therefore, we speculate that *ATP1A2* plays a regulatory role in PC. The expression of *ATP1A2* was lowest in PC-3 and DU145 cells compared with other PC cell lines, so we used them for further analyses. Previous studies have revealed that the proliferation, apoptosis, invasion and metastasis of tumor cells are the pathological basis of the development of cancers (28,29). In this study, the results showed that *ATP1A2* significantly inhibited the proliferation, migration and invasion of PC-3 and DU145 cells and promoted the apoptosis of PC-3 and DU145 cells.

EMT is a necessary process for the distant metastasis of PC (28). MMPs such as MMP-2 and MMP-9 degrade the extracellular matrix (ECM) and enhance cell invasion (30). Our results confirmed that *ATP1A2* overexpression inhibited MMP-2 and MMP-9 expression. Snail significantly regulates the EMT process, which is closely related to the loss of E-cadherin and upregulation of vimentin and

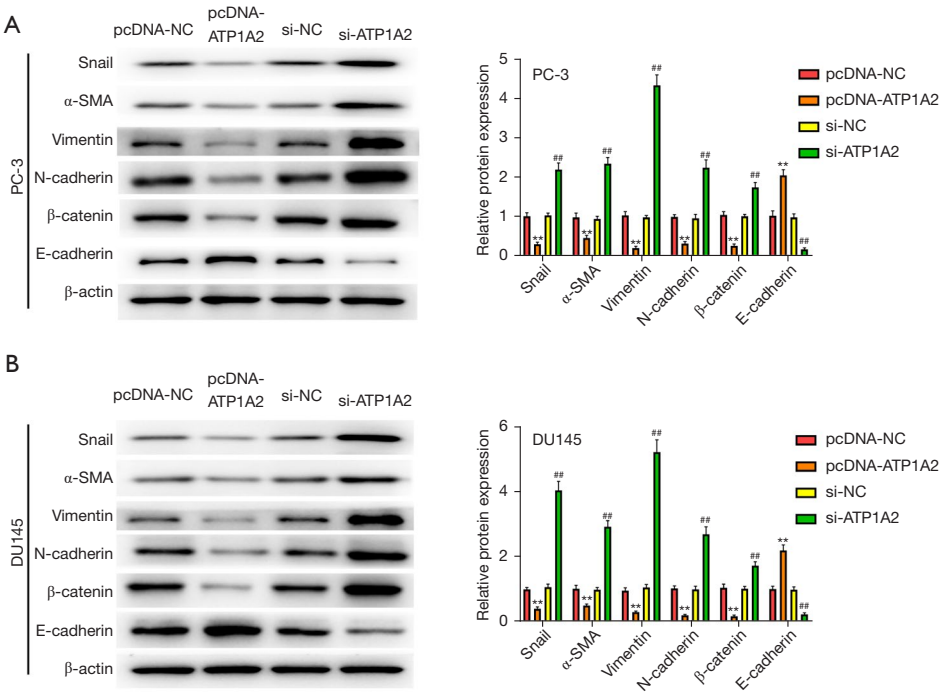

Figure 5 Effect of *ATP1A2* on EMT in PC cells. After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the expressions of Snail, α-SMA, vimentin, N-cadherin, β-catenin and E-cadherin in (A) PC-3 cells and (B) DU145 cells were determined using western blot. **, P<0.01; ##, P<0.01. EMT, epithelial-mesenchymal transition; PC, prostate cancer.

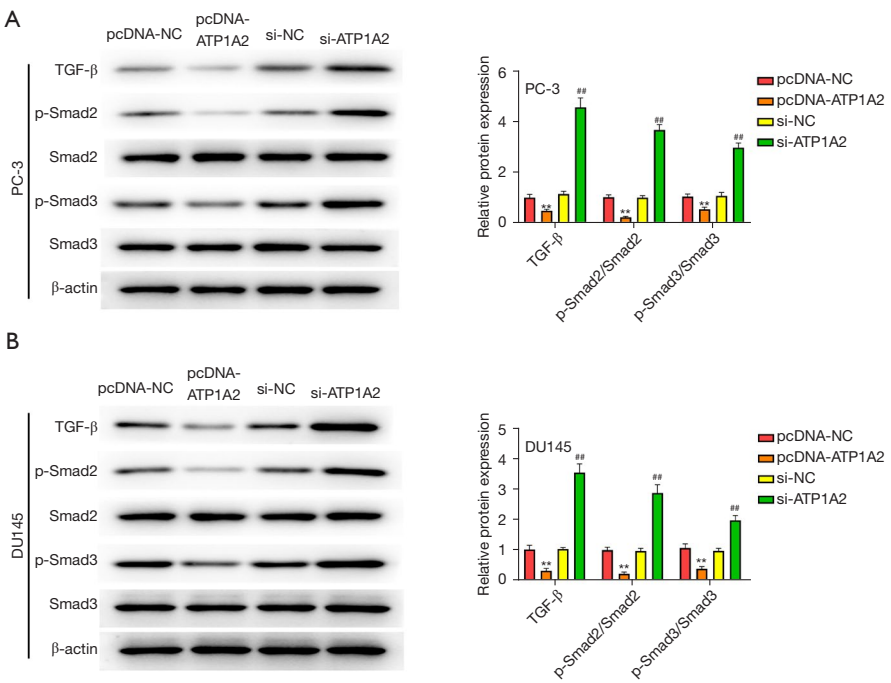

Figure 6 Effect of *ATP1A2* on the TGF-β/Smad pathway in PC cells. After transfection with pcDNA-*ATP1A2* or si-*ATP1A2*, the expressions of TGF-β, p-Smad2, Smad2, p-Smad3 and Smad3 in (A) PC-3 cells and (B) DU145 cells were determined using western blot. **, P<0.01; ##, P<0.01. TGF, transforming growth factor; PC, prostate cancer.

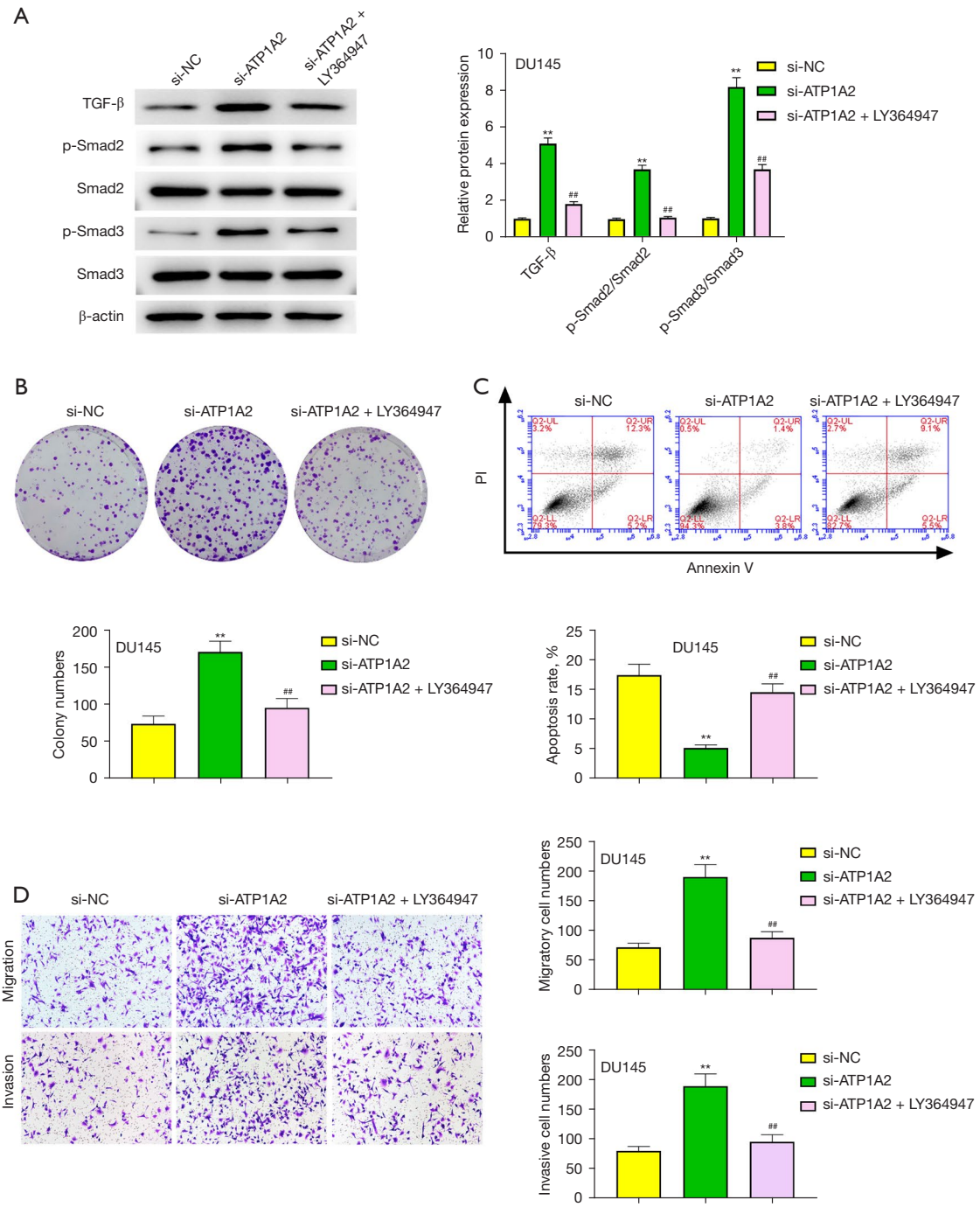

Figure 7 Effect of *ATP1A2* on the proliferation, migration and invasion, and promotion of apoptosis of PC cells through inhibition of the TGF-β/Smad pathway. (A) After transfection with si-*ATP1A2* and treatment with LY364947, the expression of TGF-β, p-Smad2, Smad2, p-Smad3 and Smad3 in DU145 cells was determined by western blot. (B) After transfection with si-*ATP1A2* and treatment with LY364947, the colony numbers of DU145 cells were detected using colony formation assay (stained with crystal violet; magnification 100×). (C) After transfection with si-*ATP1A2* and treatment with LY364947, the apoptosis of DU145 cells was analyzed using flow cytometry (stained with Annexin V-FITC and propidium iodide). (D) After transfection with si-*ATP1A2* and treatment with LY364947, the migration and invasion of DU145 cells were detected by transwell assay (stained with crystal violet; magnification 200×). **, P<0.01; ##, P<0.01. PC, prostate cancer; TGF, transforming growth factor.

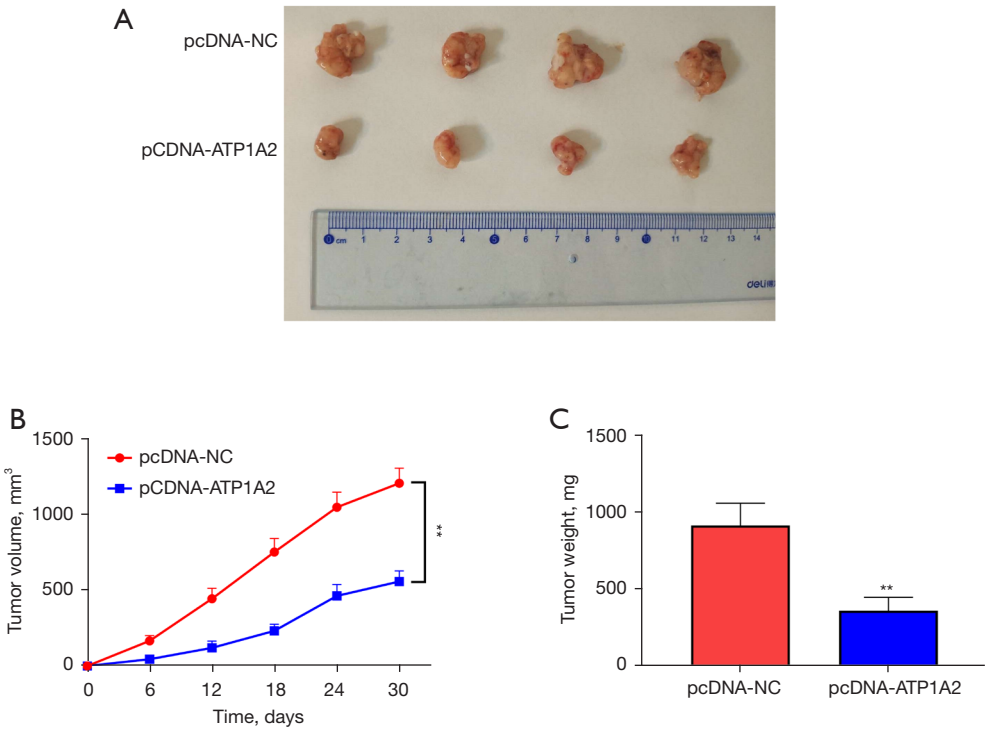

Figure 8 Effect of *ATP1A2* on tumor growth in nude mice. (A) Images of representative xenograft tumors. (B) Subcutaneous tumor volumes in the pcDNA-NC and pcDNA-*ATP1A2* groups. (C) Subcutaneous tumor weights in pcDNA-NC and pcDNA-ATP1A2 groups. **, P<0.01.

fibronectin (31-33). Cytological studies have confirmed that reduced intracellular stability of the Snail protein can inhibit the EMT process and the malignant phenotype of cancer cells (34). In this study, we confirmed that *ATP1A2* can significantly inhibit EMT-associated proteins and Snail in PC cells.

TGF-β1 is highly expressed in a variety of tumors, and its role in tumor development has been explored both *in vitro* and in transgenic mice (35). However, the role of TGF-β1 in carcinogenesis is complex. Many tumor cells become insensitive to TGF-β1-mediated growth inhibition, suggesting that increased TGF-β1 expression may alter the tumor microenvironment, such as inducing local angiogenesis, ECM generation, and inhibiting immune surveillance, which provides a favorable environment for the proliferation of tumor cells (36). In the process of tumor development, the increase in TGF-β1 synthesis plays a role in promoting tumor development (37). Malignant proliferation of cancer cells originating from the prostate epithelium lose cell polarity after EMT, resulting in decreased intercellular adhesion and enhanced potential for invasion and migration. At the same time, the expression of epithelial markers E-cadherin and tight junction protein ZO-1 is downregulated, and the expression of stromal cell markers N-cadherin and vimentin is upregulated (38,39). TGF-β is an effective inducer of EMT, which is characterized by decreased expression of E-cadherin, and upregulation of interstitial proteins (40). During cancer progression, classical Smad is a key influential factor for TGF-β-induced EMT, which is abnormally reactivated during cancer progression and can be used to regulate EMT through the interaction of related transcription factors (41). TGF-β can induce EMT by activating Smad3 and Smad2 signaling (42). In this study, we confirmed that *ATP1A2* significantly inhibited the activation of the TGF-β/Smad pathway in PC. Moreover, the rescue experiments further directly verified that the TGF-β/Smad pathway was a target of *ATP1A2*. All these results confirmed that the promotion of *ATP1A2* could be choosed for PC treatment in the future.

However, there are also shortcomings in our research. There is not only one signaling pathway that has a potential targeting relationship with *ATP1A2*. Therefore, whether *ATP1A2* affects other pathways in PC will be further

investigated in our future experiments. More work is required to explore how *ATP1A2* can be used as a novel therapeutic target for the treatment of PC.

Conclusions

In summary, we first confirmed that the expression of *ATP1A2* was decreased in PC tissues and cells. We also revealed that *ATP1A2* significantly inhibits the proliferation, migration, invasion and EMT of PC cells, and may be regulated through the TGF-β/Smad pathway. *ATP1A2* may be an effective therapeutic target for the treatment of PC.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-21-1117/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-21-1117/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-21-1117/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. KYKT2018-043-A1) granted by the Animal Ethics Committee of Affiliated Dongguan People's Hospital, Southern Medical University, in compliance with Affiliated Dongguan People's Hospital, Southern Medical University guidelines for the care and use of animals. A protocol was prepared before the study without registration.

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Cite this article as: Zhang B, Zhu Z, Zhang X, Li F, Ding A. Inhibition of the proliferation, invasion, migration, and epithelial-mesenchymal transition of prostate cancer cells through the action of *ATP1A2* on the TGF-β/Smad pathway. Transl Androl Urol 2022;11(1):53-66. doi: 10.21037/tau-21-1117

